tured with HPC and wild type + HPC transplantation). Tumor size increased significantly with the magnitude of the hepatectomy (0%, 30% and 70%) (61.74±17.92, 95.37±8.92 and 507.94±38.73mm3, P<0.05). Similarly, tumor number (1.0±0.0, 1.0±0.0 and 2.33±0.21, P<0.05) and lung metastasis was influenced by resection size (3/6, 3/6 and 6/6). Activation of HPCs could be demonstrated after 70% hepatectomy. JM1 cells co-cultured with conditioned medium from WB-F344 showed more malignant properties by increased IC50 after Adriamycin exposure, and enhanced expression of AFP, MMP9, CD133, ABCG2, and pSMAD2, β-catenin and anti-active β-catenin. Animals with JM1 tumors and concomitant WB-F344 transplantation or JM1 co-cultured with HPC displayed markedly increased tumor size, metastasis and enhanced expressions of Cyclin D1 and MMP9. Conclusion: Large resections can activate HPCs and are associated with enhanced tumor development and metastasis. Tumor cells cocultured with HPCs show malignant transformation towards more invasive capability and stem-like expression pattern. The results suggest that HPCs may play a role in HCC recurrence after surgery.

	Group A	Group B	Group C
Tumor	JM1 (WT)	JM1 (WT)	JM1 (co-culture with WB-F344)
HPC transplantation	-	+	-
Tumor volume (mm^3)	1006.7±555.9	1613.1±520.1*	1645.8±497.9*
Local metastasis	2/7	4/6	5/5*
Distant metastasis	0/7	1/6	3/5*
*P<0.05 (compared with Group A)			

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1998
DIFFERENTIAL DYNAMICS OF THE NF-KAPPAB SUBUNITS RELA AND RELB IN A MOUSE MODEL OF CHRONIC HEPATITIS B

Yasunari Nakamoto[1,2], Taro Yamashita[2], Shuichi Kaneko[2]; [1]Second Department of Internal Medicine, University of Fukui, Fukui, Japan; [2]Disease Control and Homeostasis, Kanazawa University, Kanazawa, Japan

BACKGROUND: Hepatocellular carcinoma (HCC) is a common complication of chronic viral hepatitis. In an effort to clarify the carcinogenic potential, we have developed a model of chronic immune-mediated liver disease using hepatitis B virus (HBV) transgenic mice. The transcription factor NF-kappaB family members activated by inflammatory cytokines like TNF-alpha have been implicated during liver injury and inflammation. The current study was designed to determine the dynamics of NF-kappaB subunits correlated with the increasing procarcinogenic potentials of liver tissues in the model of chronic hepatitis B. METHODS: Three-month-old HBV transgenic mice were immunologically reconstituted with bone marrow cells and splenocytes from syngeneic nontransgenic donors. Liver tissues were obtained every three months until 18 months at which time all mice developed multiple liver tumors. To assess the procarcinogenic potentials, oxidative DNA damage was immunohistochemically assessed by the formation of 8-OHdG and 4-HNE, and hepatocyte turnover was quantified by PCNA expression. Gene expression profiles were determined at mRNA (a microarray system and a real-time RT-PCR assay) and protein (Western blot analysis) levels, and the phosphorylation was estimated. RESULTS: In a microarray and an RT-PCR assay, the NF-kappaB family was seen to be induced at the earliest time point (3 months) after the onset among the oxidative stress-related signaling pathways. At protein level, the NF-kappaB subunit p65/RELA was activated at 3 months and remained elevated for the whole process of disease. Simultaneously, expression of TRAF-2 was induced and phosphorylation of IkappaB-alpha (Ser32) was reduced, leading to the p65/RELA activation. In contrast, the RELB subunit was elevated at 9 mouths and further induced in tumor tissues together with p100, a molecule functioning as a RELB-specific inhibitor. 8-OHdG and 4-HNE formation and proportions of PCNA-positive hepatocytes were increased during the progression of chronic liver disease. To evaluate the functional properties of gene expression, the activation of RELB-dependent pathway (TRAF3, NIK, RELB, p100 and p52) was detected following TNF-alpha stimulation in hepatoma cell lines HepG2 and PLC/PRF/5. CONCLUSIONS: Chronic immune-mediated hepatitis differentially enhances the NF-kappaB subunits in which the RELA-dependent pathway is activated during persistent inflammation and subsequently the RELB-dependent pathway is induced with the increasing procarcinogenic potentials, suggesting that the RELB pathway may provide potent therapeutic targets in the development of tumor microenvironment in chronic liver disease.

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MECHANISM OF THE PATHOGENESIS OF HEPATOCELLULAR CARCINOMA DURING CHRONIC ADMINISTRATION OF ETHANOL IN MICE

Mutsumi Tsuchishima, Joseph George, Hisakazu Siroeda, Takashi Saito, Tomiyasu Arisawa, Mikihiro Tsutsumi; Gastroenterology, Kanazawa Medical University, Ishikawa, Japan

Epidemiological evidence indicates that chronic intake of alcohol increases the risk of carcinogenesis in liver and gastrointestinal tract. However, the mechanism of ethanol induced hepatocarcinogenesis is not clear. In order to elucidate the effects of ethanol on the pathogenesis of hepatocellular carcinoma (HCC), male ICR mice were administered ethanol through drinking water over a period of 60 and 70 weeks at a concentration of 5% on the first week, 10% during the next 8 weeks, and 15% thereafter. The control group received equal amount of water without ethanol. Some of the control and treated mice were sacrificed at 60 weeks and the remaining at 70 weeks. At 60th week, 40% of ethanol group had visible white nodules (5-10 mm) in the liver, while such nodules were totally absent in control mice. At 70th week, several larger nodules (5-22 mm) were present in the livers of 50% mice in ethanol group. In control group, one mouse (10%) developed a single nodule. All nodules were histologically trabecular HCC composed of eosinophilic and vacuolated cells. In the livers of both control and ethanol group, a few foci were present with cellular aberration. The size of the foci in ethanol group was significantly larger than those in the control group. In order to obtain an insight of the mechanism of pathogenesis of ethanol induced HCC, immunohistochemistry was performed in paraffin embedded liver sections of control and ethanol treated mice for cytochrome P4502E1 (CYP2E1), 4-hydroxy-nonenal (HNE), a marker for reactive oxygen species (ROS), and c-Myc. There was dramatic upregulation of all these molecules in the foci where cellular aberration was present. The results indicated that chronic administration of ethanol upregulates CYP2E1 which results in the production of ROS that in turn induces c-Myc and triggers the development of HCC in mice. Our data

suggest that ethanol acts as a promoter for the pathogenesis of HCC.

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2000
GALNT1 IS DOWN REGULATED IN HEPATOCELLULAR CARCINOMA AND ITS EXPRESSION REGULATES EGF AND VEGF SIGNALING

Yao-Ming Wu[1], Miao-Juei Huang[2], Min-Chuan Huang[2]; [1]Surgery, National Taiwan University Hospital, taipei, Taiwan; [2]Graduate Institute of Anatomy and Cell Biology, National Taiwan University College of Medicine, taipei, Taiwan

Purpose: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide. It is well acknowledged that receptor tyrosine kinases (RTKs), including EGFR, VEGFR, and c-Met, play critical roles in HCC progression. In addition, there is significant evidence correlating glycosylation to disease development. N-acetylgalactosaminyltransferase 1 (GALNT1) is a member of the 20 GALNT enzymes that catalyzes the transfer of N-acetylgalactosamine (GalNAc) to serine or threonine residues in a protein, which is the first step of O-glycosylation. The purpose of this study is to investigate the role of GALNT1 in regulation of RTK signaling and its subsequent effects in HCC cells. Method: The expression level of GALNT1 in paired HCC tissues and HCC cell lines were analyzed by real-time PCR and western blotting. Knockdown of GALNT1 in PLC5, HepG2, and Hep3B was achieved using GALNT1 siRNA. Subsequent Matrigel invasion assays were performed and western blot analysis was used to elucidate signaling pathways after treatment of EGF 20 ng/ml, VEGF 10 ng/ml, and HGF 50 ng/ml, respectively. Results: Real-time PCR revealed a decreased level of GALNT1 expression in the matched primary HCC compared to its normal tissue. EGF- and VEGF-induced invasion was enhanced in the GALNT1 silenced PLC5 and Hep3B cells. Western blot analysis revealed that GALNT1 knockdown increased the expression level of EGFR in all three cell lines and enhanced the subsequent phosphorylation of AKT and Erk1/2. VEGF treatment also yielded a similar pattern of enhanced downstream signaling. In contrast, HGF treatment did not affect the phosphorylation of both AKT and Erk1/2 in all three cell lines. Conclusion: GALNT1 expression is dysregulated in HCC and its decreased expression enhances HCC cell invasion possibly via EGF and VEGF signaling. These findings provide a novel insight into the molecular pathogenesis of HCC.

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2001
THE ROLE OF THE UNFOLDED PROTEIN RESPONSE IN HEPATIC CARCINOGENESIS

Viviana Scaiewicz[2], Avital Nahmias[2], Liran Barda[2], Emmanuelle Merquiol[2], Boaz Tirosh[3], Oren Shibolet[1]; [1]Liver Unit, Department of Gastroenterology and Liver Disease, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; [2]Liver Unit Division of Internal Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [3]Institute for Drug Research, School of Pharmacy, Faculty of Medicine, Hebrew University in Jerusalem, Jerusalem, Israel

Background: The endoplasmic reticulum (ER) is responsible for protein folding and modification. ER stress occurs when the amount of protein entering into the ER exceeds its folding capacity, inducing a cyto-protective reaction collectively termed the unfolded protein response (UPR). Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer-related death worldwide. ER stress has been suggested to play a potential role in hepatic pathology and chronic inflammation. However, its role in HCC has not been addressed. Aim: We hypothesized that ER Stress is activated during hepatic carcinogenesis, and may play an important role in cancer initiation and/or progression. Methods: We used quantitative real-time PCR analysis and western blotting on total RNA and protein respectively, isolated from liver tumors and parenchyma of DEN, a chemically induced, HCC mouse model. Immunohistochemistry staining for UPR proteins was performed on human tumor tissue collected from the Hadassah-Hebrew University Medical Center tissue bank. The research was approved by the institutional review board. Results: we observed a 3-fold increase in the spliced form of XBP1 mRNA and a 6-fold increase of CHOP mRNA in tumors, compared to un-involved parenchyma. Downstream UPR target genes including ERDJ4 and p58ipk showed only a mild elevation, which may suggest a partial or aborted activation of the UPR. Immunohistochemistry staining results from DEN-induced tumors showed that XBP1 and CHOP were localized to the nucleus of cancerous cells, indicating activation. Sections from human lung, colon and hepatocellular carcinomas were also analyzed by Immunohistochemistry staining and showed that in all analyzed samples,CHOP was located in the nucleus of tumor cells. Interstingly, despite the known pro-apoptotic role of CHOP, we found no activation of apoptosis in the tumors, as assessed by activated caspase-3 staining. ATF6, another UPR activator was also localized to the nucleus of cancerous cells, while no nuclear staining was observed in un-involved parenchyma. Conclusions: We found that ER stress occurs and the UPR is partially activated in tumor tissue from chemically induced HCC mouse model and human carcinomas, but not in un-involved parenchyma. The physiologic role of this activation is currently unclear. Activation of the UPR in HCC may mark this pathway as a possible target for therapeutic intervention.

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2002
S-ADENOSYLMETHIONINE REGULATES UBIQUITIN-CONJUGATING ENZYME 9 EXPRESSION AND SUMOYLATION: MOLECULAR MECHANISM AND IMPLICATION IN HEPATOCARCINOGENESIS

Maria Lauda Tomasi[1], Komal Ramani[1], Tony W. Li[1], Jun Xu[2], Jose M. Mato[3], Shelly Lu[1]; [1]Medicine, Keck School of Medicine USC, Los Angeles, CA; [2]Pathology, Keck School of Medicine USC, Los Angeles, CA; [3]CIC bioGUNE, Derio, Spain

BACKGROUND&AIMS: Ubiquitin-conjugating enzyme 9 (Ubc9) is an E2-conjugating enzyme that transfers the activated small ubiquitin-like modifier (SUMO) to protein substrates and is critical in sumoylation-mediated cellular pathways. Genotoxic stress induces sumoylation of numerous proteins. Ubc9 is overexpressed in several malignancies, such as melanoma and lung adenocarcinoma, but its expression in hepatocellular carcinoma (HCC) has not been reported. Chronic hepatic S-adenosylmethionine (SAMe) deficiency occurs in methionine adenosyltransferase 1A (Mat1a) knockout (KO) mice, which exhibit increased genotoxic stress and predisposition to liver injury and malignant transformation. Hepatic SAMe level falls also during intragastric ethanol infusion in mice. At the AASLD last year we showed that Ubc9 expression is up-regulated in livers of Mat1a KO and ethanol fed mice and human HCC.